BIOMEDICAL RESEARCH FOUNDATION, ACADEMY OF ATHENS (BRFAA) - www.bioacademy.gr

RESEARCH DIRECTIONS

CARDIOVASCULAR

- Development
- Physiology - Pathology
- Cellular Death

NEUROBIOLOGY

- Development
- Neurodegenerative Diseases
- Memory and Learning

BIOLOGY OF STRESS

- Metabolic Diseases
- Diabetes Obesity
- Systemic Responses

REGULATION OF GENE EXPRESSION

- Basic Molecular Mechanisms
- Gene Expression Networks
- Genetic re-programming

GENETICS OF AGEING

- In C. elegans
- Telomeres
- Systemic analysis

INFLAMMATION-TISSUE REMODELING

- Severe Asthma
- Chronic Obstructive Pulmonary Disease
- Interstitial Lung Diseases
- Renal Failure
- Rheumatoid Arthritis
- Ulcerative Colitis, Crohn's Disease

CANCER BIOLOGY

- Mechanisms of carcinogenesis
- Novel Markers
- Cutting edge methodologies for:
- Treatment and diagnosis
- Gene Therapy
- New drugs

TransMed

translational medicine

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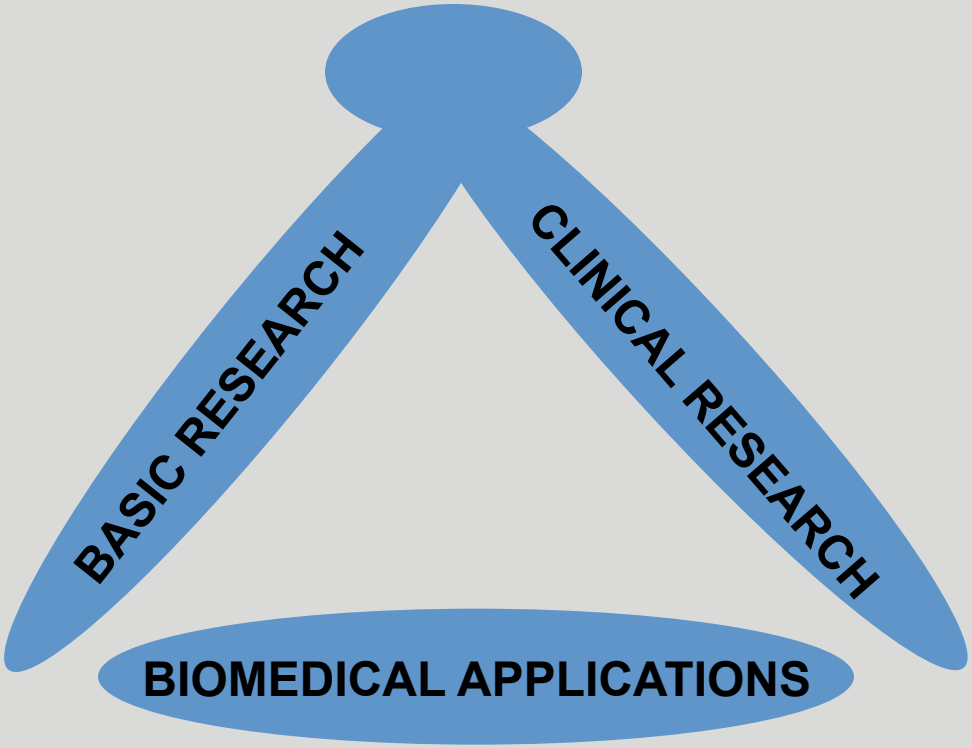
Prof. Jonathan Seckl

Dimitris Kalamatianos
Sevi Karaliota
Charalambos Sigalas
Alexia Polissidis
Panagiotis Tsakanikas

MAIN MISSION OF BRFAA :
THE CATALYSIS OF TRANSLATIONAL RESEARCH



CENTER FOR CLINICAL RESEARCH

Cardiovascular Disorders	⟷	Onassis Cardiac Surgery Center, Attikon Hospital, Ippokrateio Hospital, Agia Sofia Children's Hospital, University of Thessaly Hospital, ΑΧΕΠΑ Hospital in Thessalonica, University of Thrace Hospital
Metabolic Diseases	⟷	Agia Sofia Children's Hospital (24000 patients / year), Evaggelismos Hospital
Hematological Diseases		
Positron Emission Tomography		
Neurodegenerative Diseases	⟷	Attikon Hospital, University of Thessaly Hospital, University of Patras
Respiratory Diseases	⟷	Evaggelismos Hospital, Sotiria, Sismanoglio, Attikon Hospital, Ippokrateio Hospital, University of Thessaly Hospital, ΑΧΕΠΑ Hospital in Thessalonica, University of Thrace Hospital, University of Patras, University of Crete, University of Ioannina etc.

Positron emission Tomography

6 semi-intensive care Patient beds

Echocardiography Facility

CENTER FOR EXPERIMENTAL SURGERY

A team of investigators performing a heart operation in a pig, in one of the three Surgical Suites of the Centre.

Fully equipped surgery units capable of supporting broad range of surgical procedures (>2000) in animal models (mice, rats, rabbits, pigs).

Intensive Care Unit for post operative monitoring and support of the experimental animals

Medical Imaging Unit, analyses with the aid of State-of the – Art methodology such as X-Ray, Ultrasound, Computer and Magnetic Resonance Tomography.

ANIMAL HOUSE

Highly trained animal caretaker handling experimental animals in IVF cages

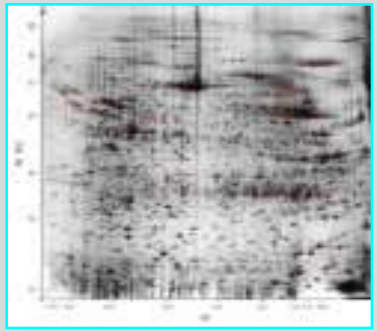
1600 m2, supporting ~8.000 mice and rats, 70 rabbits and 20 pigs.

SPF conditions in individually ventilated cages. Animals housed under the guidelines of the European Convention 123/Council of Europe and Directive 86/609/EEC), «AAALAC Int.», and «FELASA».

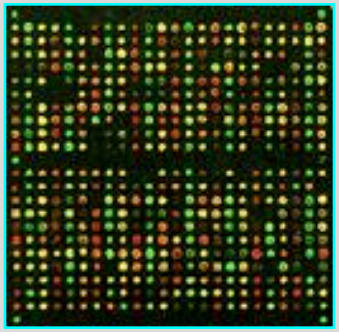
Soon the capacity of the Animal House will increase to 16.000. Within the next two years a new animal house will raise the capacity to 80.000 mice.

SUPPORT FACILITIES AT BRFAA

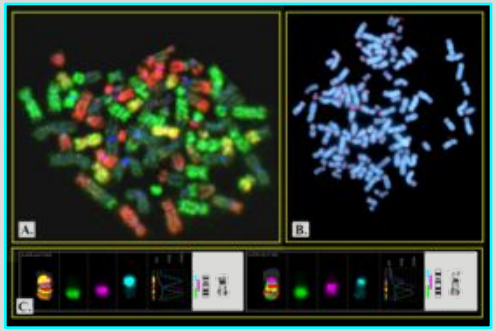
PROTEOMICS



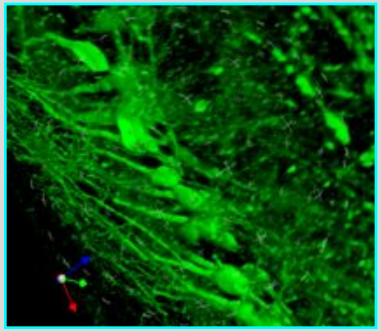
GENOMICS



CYTOGENETICS



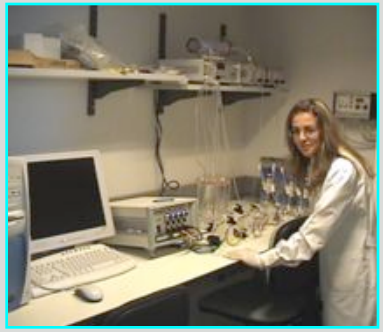
BIOIMAGING



Laser Micro-dissection

Transgenics

FACS-SORTING



Lung Function Measurements

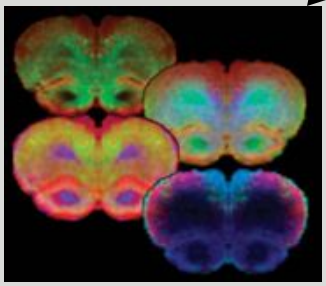
Proteomics

Large-scale analysis of proteins

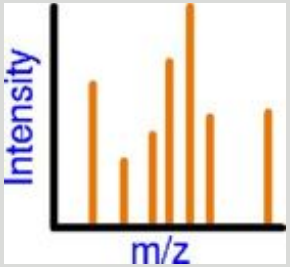
Image Prep

MALDI TOF-TOF Autoflex (Bruker)

Orbitrap (Thermo)



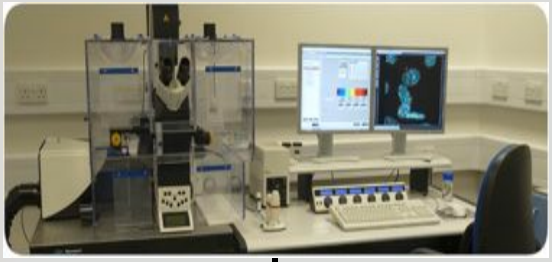
Tissue Imaging



Protein Identification, Quantitation,
Post Translational Modification

BioImaging Facility

Inverted Confocal and
Live Cell imaging System
LEICA SP5



Calcium
photometry/imaging

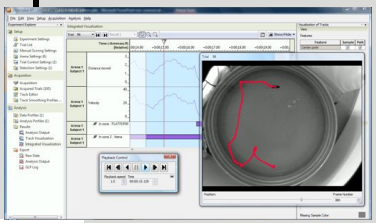
Two Upright Imaging Microscopes
With
CCD Cameras

Three Inverted Imaging Microscopes
With
CCD Cameras

NETWORK

Upright Confocal
And
Deconvolution Workstation
LEICA SP5 II

Neurolucida and
Stereo Investigator
Workstation
with Colour CCD Camera



Noldus Animal Behaviour
Analysis System

Fluorescence Dissection Microscope with Camera

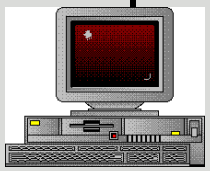
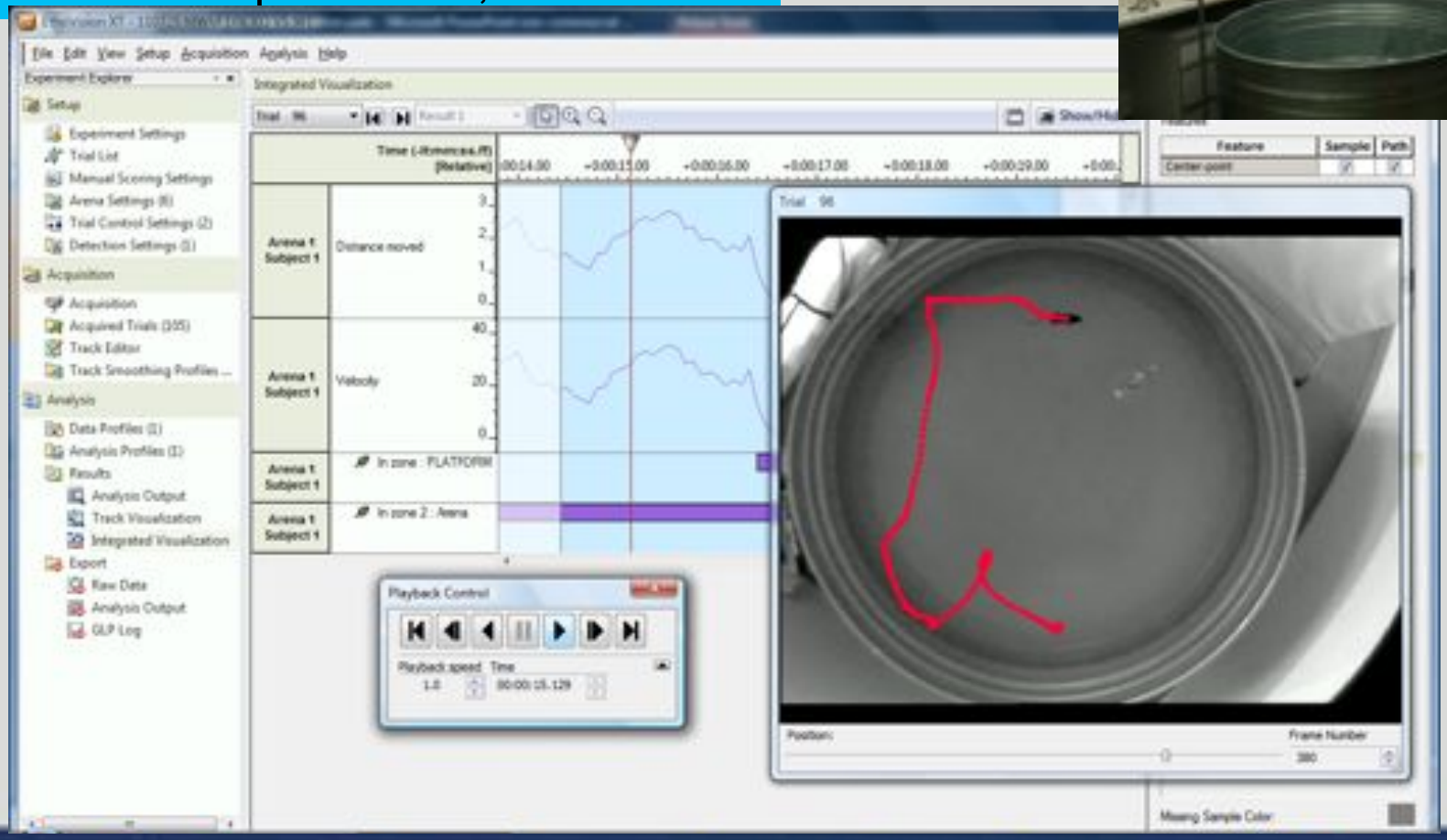


Image Analysis Workstations

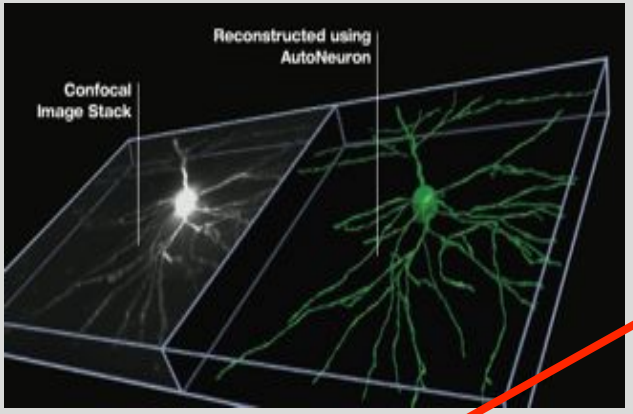
NAS Server

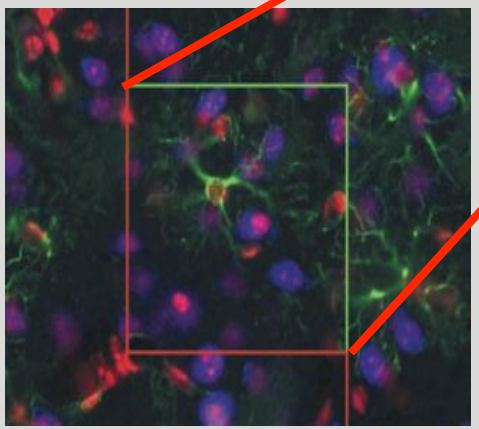
Noldus Ethovision XT8

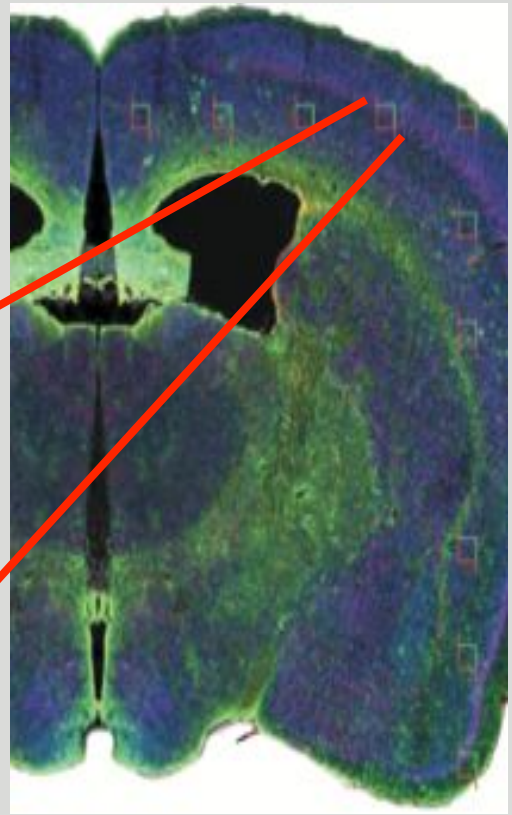
- Video tracking software for behaviour experiments
  - Morris water maze, open field, elevated plus maze, etc



Stereology Workstation for unbiased Counting and Volumetric Data Measurement







STEREO INVESTIGATOR
Unbiased Stereological measurements:
Quantify cell counts, lengths, areas and volumes.

NEUROLUCIDA
Neuro-anatomical analysis system for Neuron Tracing, 3D mapping, Image Analysis and Morphometry.

Target Identification (PD Patients)

- 1 Target: Familial Mutations Identified
- An Additional Target is Explored

In vivo Relevance (Animal Models)

- Created PD Animal Models in Mice

Preclinical In vivo Efficacy

- Pharmacokinetics
- Compound Metabolism
- Disease Modification

Molecular Mechanism (Cell Lines)

- Neuroprotective Effects in Cell Lines
- Characterized Molecular Mechanism

Target Modulation (Chemistry)

- *In Silico* Chemistry
- Medicinal Chemistry
- Brain Penetration
- Lead Compound identified

Neurodegenerative diseases (1)

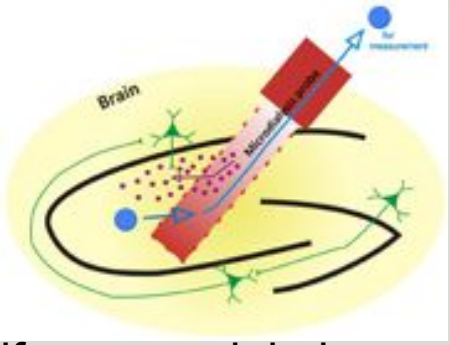
A. Establishment of collaboration between BRFAA and Lund University

B. **Transfer of knowledge** for :

a. Production and delivery of high purity viral vectors in rodent brain (mouse, rat)
b. Use of such vectors for the generation of PD animal models
c. Screening of potential therapeutic targets
d. Identification of Lamp2a as a target for Parkinson's disease (Xilouri et al, Brain 2013)

Use of:
Neurolucida and StereoInvestigator Software to accurately visualize and quantify neuronal cells in tissues

Neurodegenerative diseases (2)



1. Biomarker search

- Development of novel, ultra-sensitive ELISA assays to quantify a-synuclein in biological fluids (CSF, blood plasma and serum, ISF) and media from cell culture (iPS cells – Collaborations with University of Oxford and Hellenic Pasteur Institute)

2. Validation of therapeutic compounds using *in vivo* microdialysis in PD mouse models

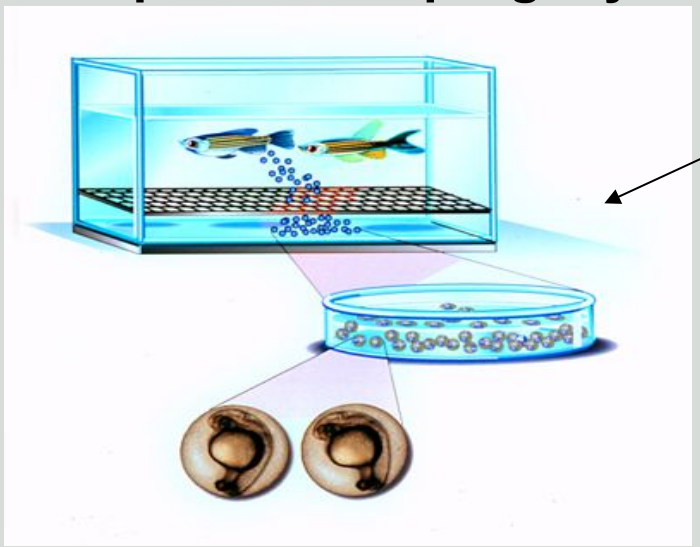
- Assessment of the immunobinding efficacy of a new antibody to be used for PD immunotherapy - Collaboration with BIOGEN Idec.
- Assessment of the ability of a new compound to reduce the levels of extracellular a-synuclein in the brain – Collaboration with reMYND (Belgium)

3. Looking at the extracellular space using in vivo microdialysis and reverse microdialysis

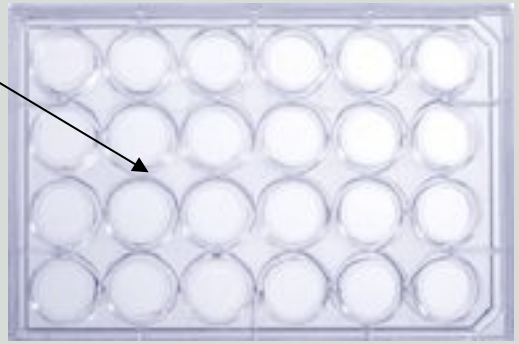
- Investigation of cellular secretory pathways in mouse brain
- Characterization of new PD mouse models (synuclein BAC mouse, Collaboration with University of Oxford)

Zebrafish offer an alternative strategy for drug discovery (phenotype-driven instead of target-driven)

external fertilization
plethora of progeny

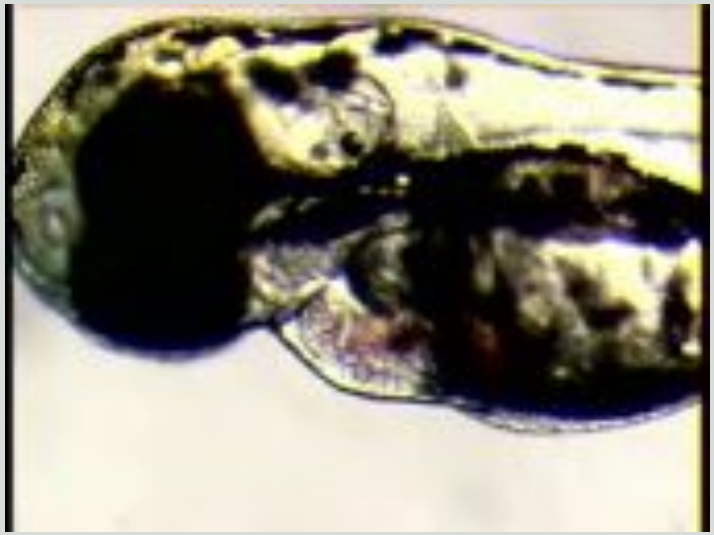


Add the chemical of your choice

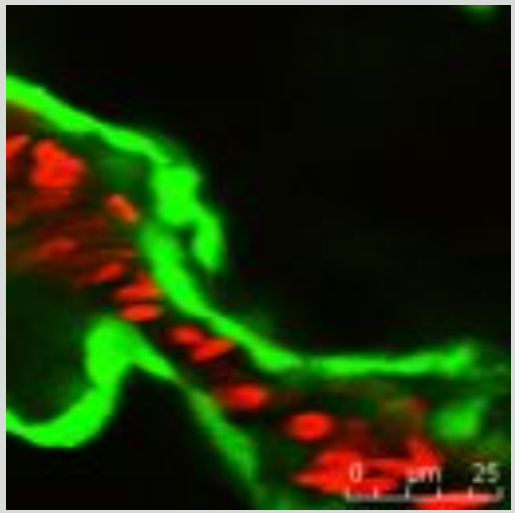


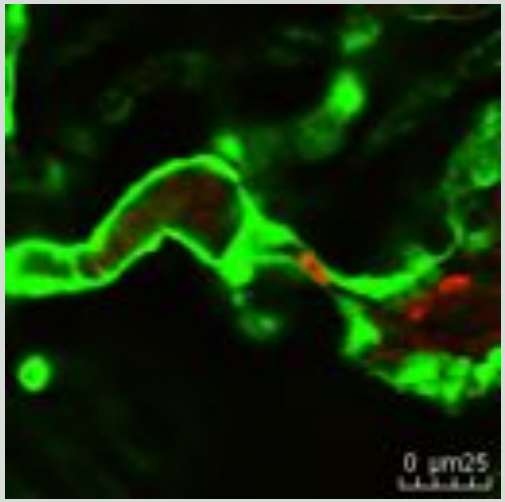
>20 embryos per well for 5 days (small volume, statistically significant numbers)

Arrhythmic fish / Long QT test

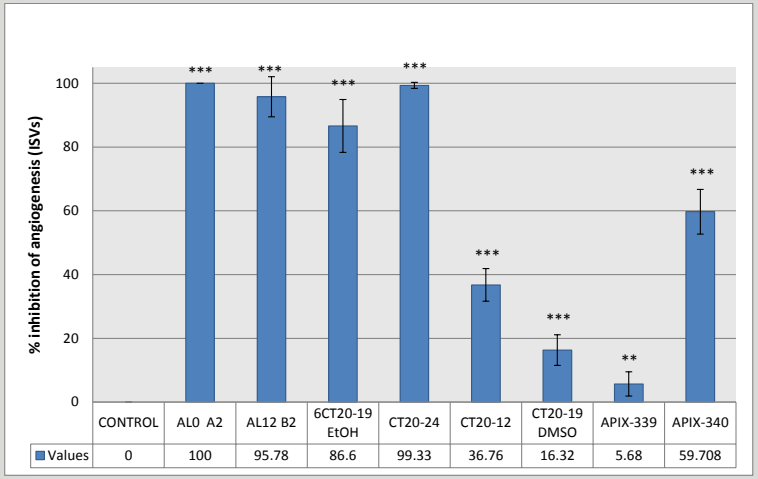


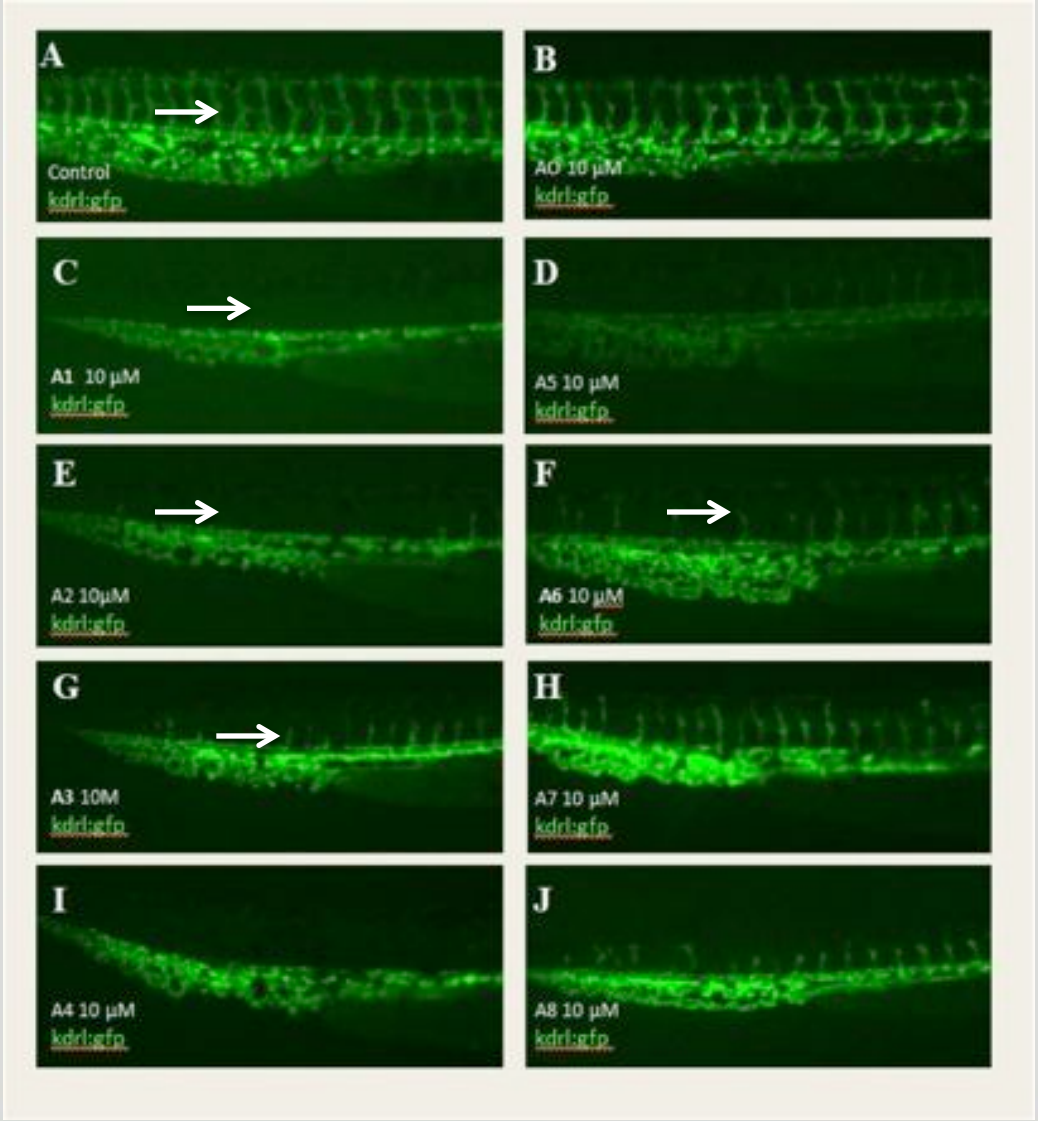
Heart function at the cellular level *in vivo*
Aortic stenosis

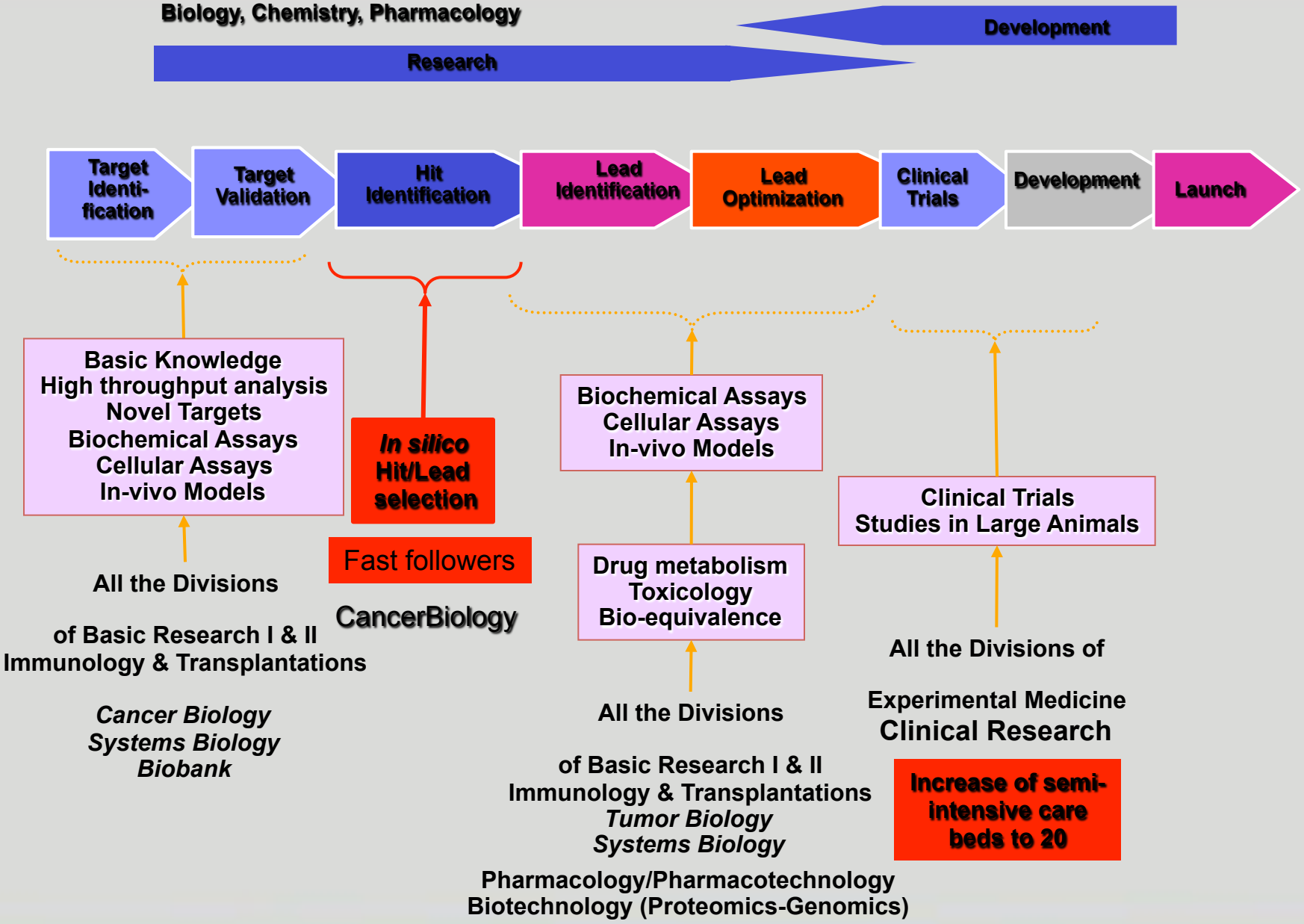




An *in vivo* phenotypic screen of 90 anthranilamide analogues gave 7 new antiangiogenic molecules






Biology, Chemistry, Pharmacology
Development
Research
Target Identi-fication
Target Validation
Hit Identification
Lead Identification
Lead Optimization
Clinical Trials
Development
Launch
Basic Knowledge
High throughput analysis
Novel Targets
Biochemical Assays
Cellular Assays
In-vivo Models
All the Divisions
of Basic Research I & II
Immunology & Transplantations
Cancer Biology
Systems Biology
Biobank
In silico Hit/Lead selection
Fast followers
CancerBiology
Biochemical Assays
Cellular Assays
In-vivo Models
Drug metabolism
Toxicology
Bio-equivalence
All the Divisions
of Basic Research I & II
Immunology & Transplantations
Tumor Biology
Systems Biology
Pharmacology/Pharmacotechnology
Biotechnology (Proteomics-Genomics)
Clinical Trials
Studies in Large Animals
All the Divisions of
Experimental Medicine
Clinical Research
Increase of semi-intensive care beds to 20

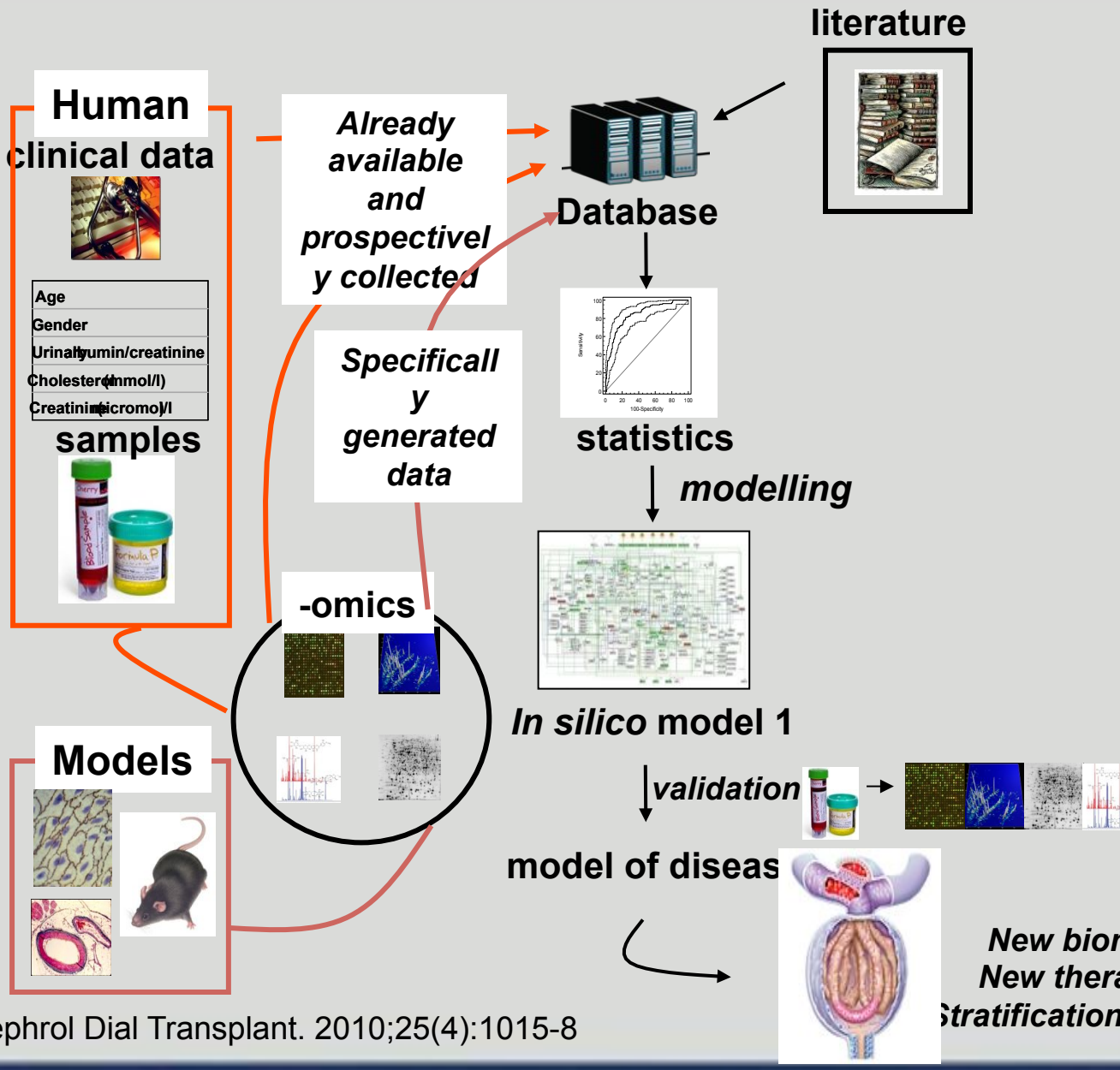
DIVISION OF PHARMACOLOGY - PHARMACOTECHNOLOGY

The lab is equipped with Hybrid system of Liquid Chromatography – Mass Spectroscopy of triple quadrupole – Ion trap (LC-MS/MS), and all the other equipment that are required to perform bioanalysis and validation of drugs.

The laboratory operates according to the Good Laboratory Practice (GLP) standards and has been awarded an ISO/IEC 17025:2005 certificate.

- Unknown compound analysis (Q1 MS, MS/MS, MS/MS/MS)
- Qualitative/ Quantitative analysis of drugs in biological fluids/tissues
- Pharmacokinetics (PK) of drugs in pre-clinical/clinical stages
- Drug metabolism
- Bioavailability and bioequivalence studies
- Quantitative determination of biomarkers (*e.g.* peptides, proteins) in various matrices (*e.g.* plasma)

Biomarker discovery: integrative approach



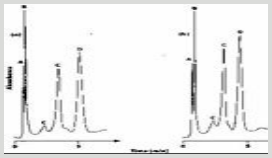
Modified from Nephrol Dial Transplant. 2010;25(4):1015-8

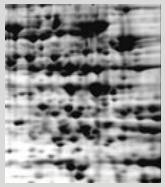
Discovery of biomarkers : Experimental workflow

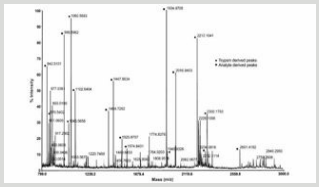
Urine

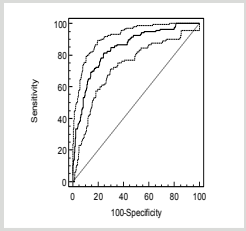
Models

Proteomics







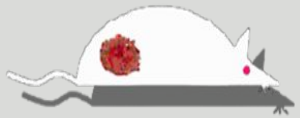


Statistical association

biological process
molecular function
cellular component

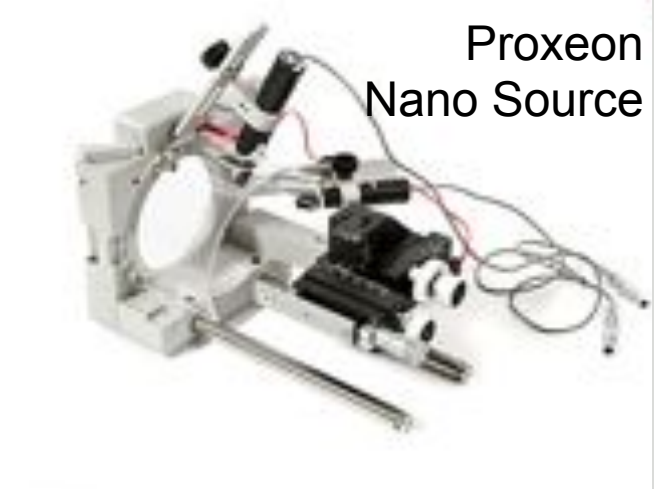
Verification
Clinical samples

Functional studies



Beyond the State-of-the-Art

- The Pharmacology – Pharmacotechnology Laboratory is moving beyond the state-of-the-art by acquiring the newer NanoSpray technology which will upgrade all analyses.
- The low-flow interface (nL/min) will allow increased sensitivity while the high pressure nanoLC system will afford higher chromatographic separation/resolution



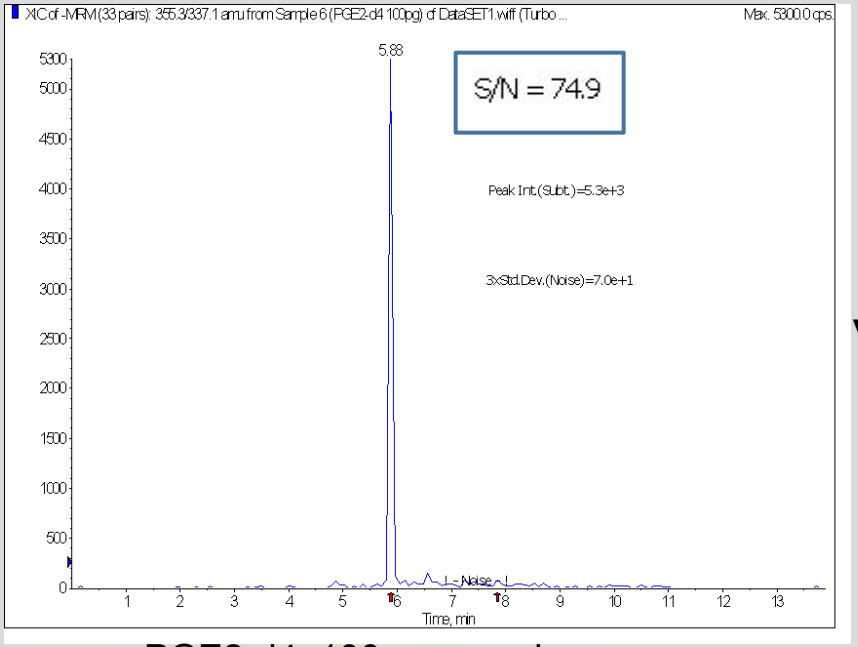
Proxeon
Nano Source

UltiMate 3000 RSLCnano Rapid Separation Nano LC System

Technological Advancements

- Installation of the Nano-Spray Technology in Pharmacology lab allowed an increase in sensitivity of 100-fold.

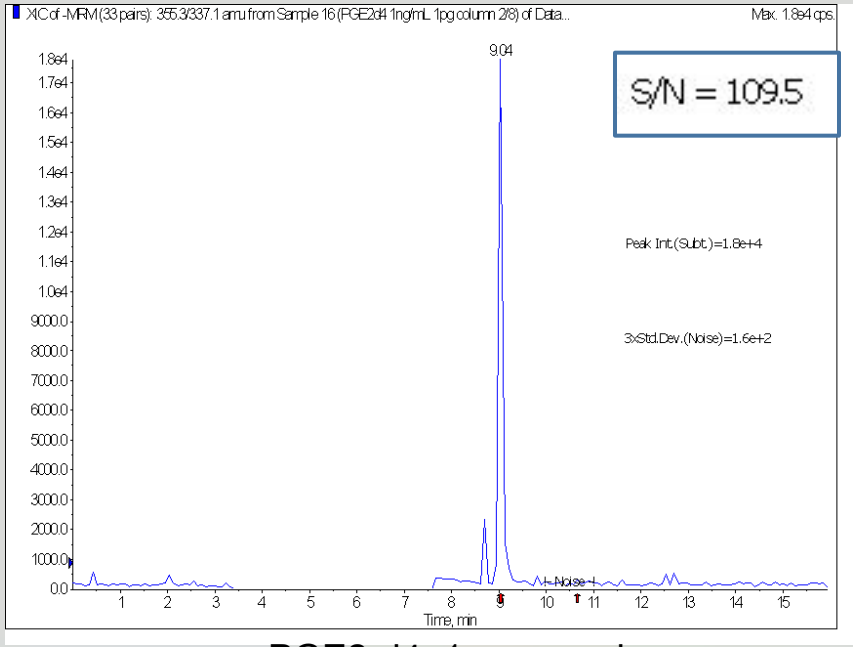
microLC-MRM



PGE2-d4 100 pg on column

Vs.

nanoLC-MRM



PGE2-d4 1 pg on column

The Animal Behavior Facility

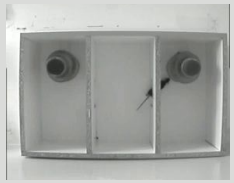
Behavioral phenotyping applications

- Genetic manipulations
- Ageing
- Potential pharmaceuticals

- **Ultimate goal: translational medicine**
  - multifaceted approach to behavioral testing in animal models of human disease

Available behavioral tests (1)

Behavioral index	Equipment
Locomotor activity	Open field with video tracking software
Motor coordination & balance eg. Parkinson's disease	Rotarod Pole test Cylinder test Beam traversal Footprinting Grip strength
Pain assessment	Hot plate
Social behavior	Sociability chamber



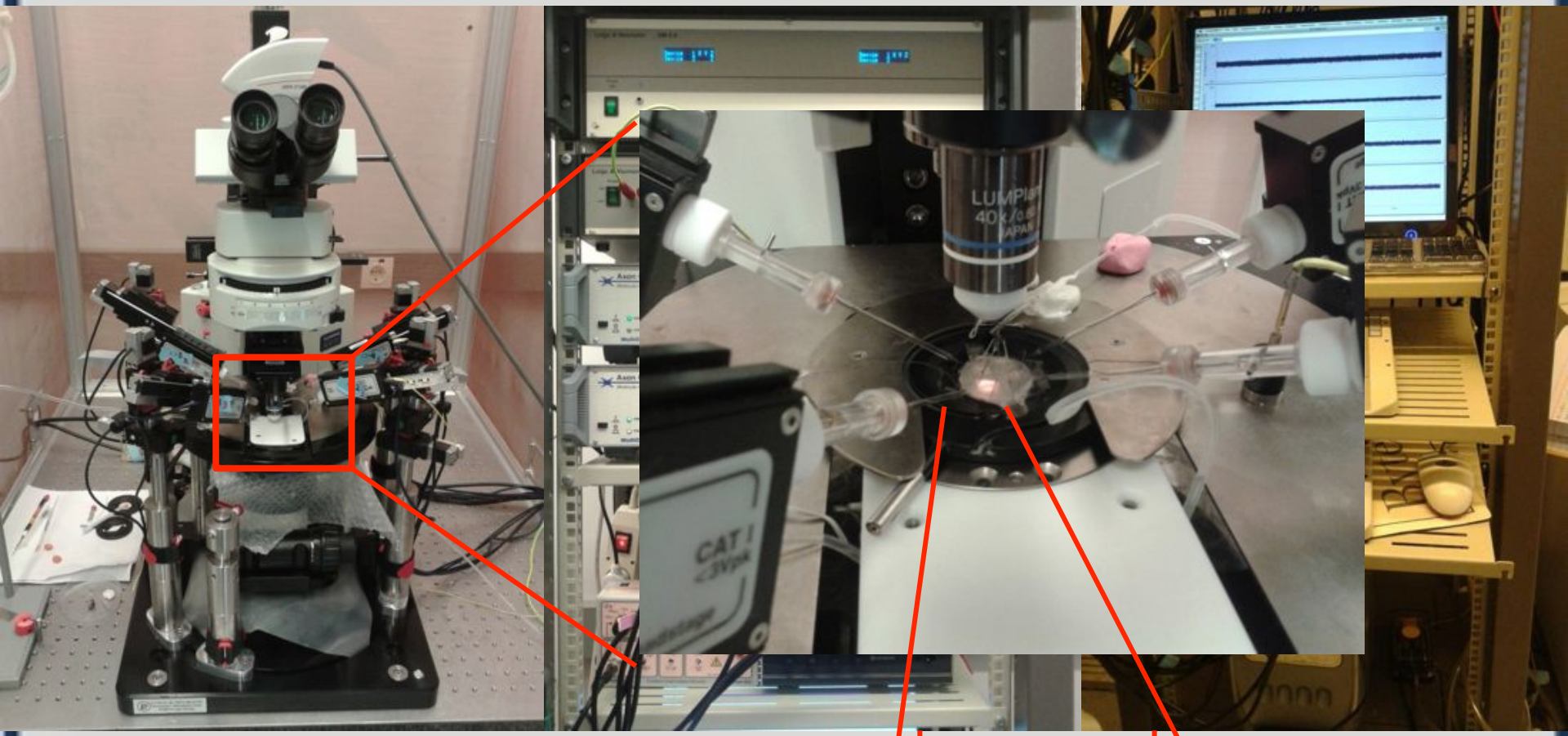
Available behavioral tests (2)

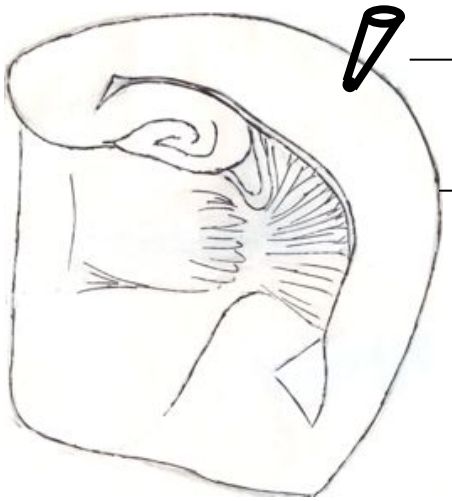
Behavioral index	Equipment
Anxiety	Elevated plus maze Light dark box Open field (center vs. perimeter)
Depression	Forced swim test Tail supsension
Learning & memory eg. Alzheimer's disease	Active/passive avoidance Object recognition/location Morris water maze 8-arm radial maze
Attention & sensorimotor gating eg. schizophrenia	Acoustic startle chamber

ELECTROPHYSIOLOGY

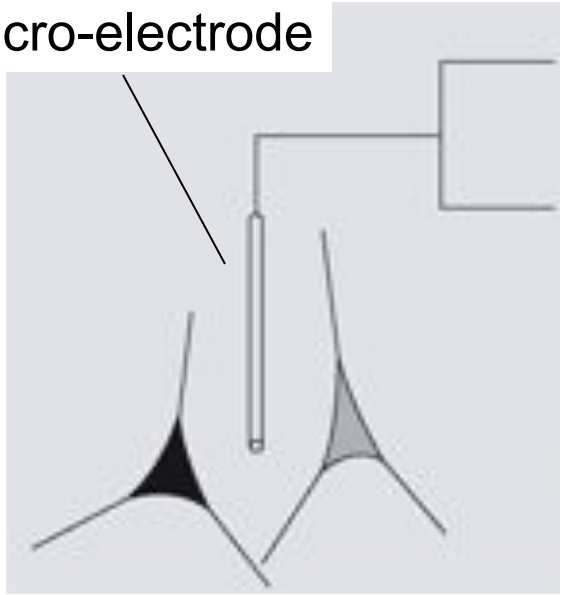
- Study of the electrical properties of molecules, cells, tissues or organs.
- Measures voltage changes or electric current to make inferences about function
- Can study physiological and pathological function of biological systems over a wide spectrum, ranging from single ion channels (e.g. nicotinic Acetylcholine receptor) to whole organs (e.g. brain, heart etc.)
- Drug assessment

ELECTROPHYSIOLOGY
equipment

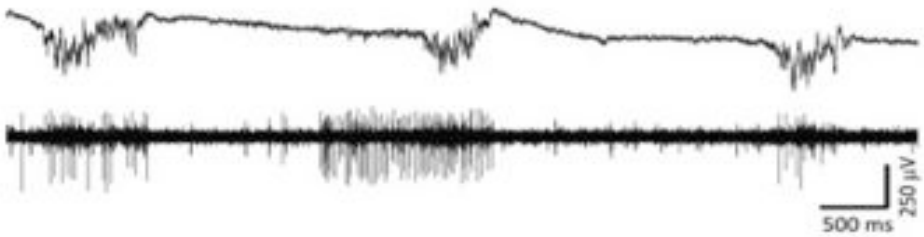




Micro-electrode



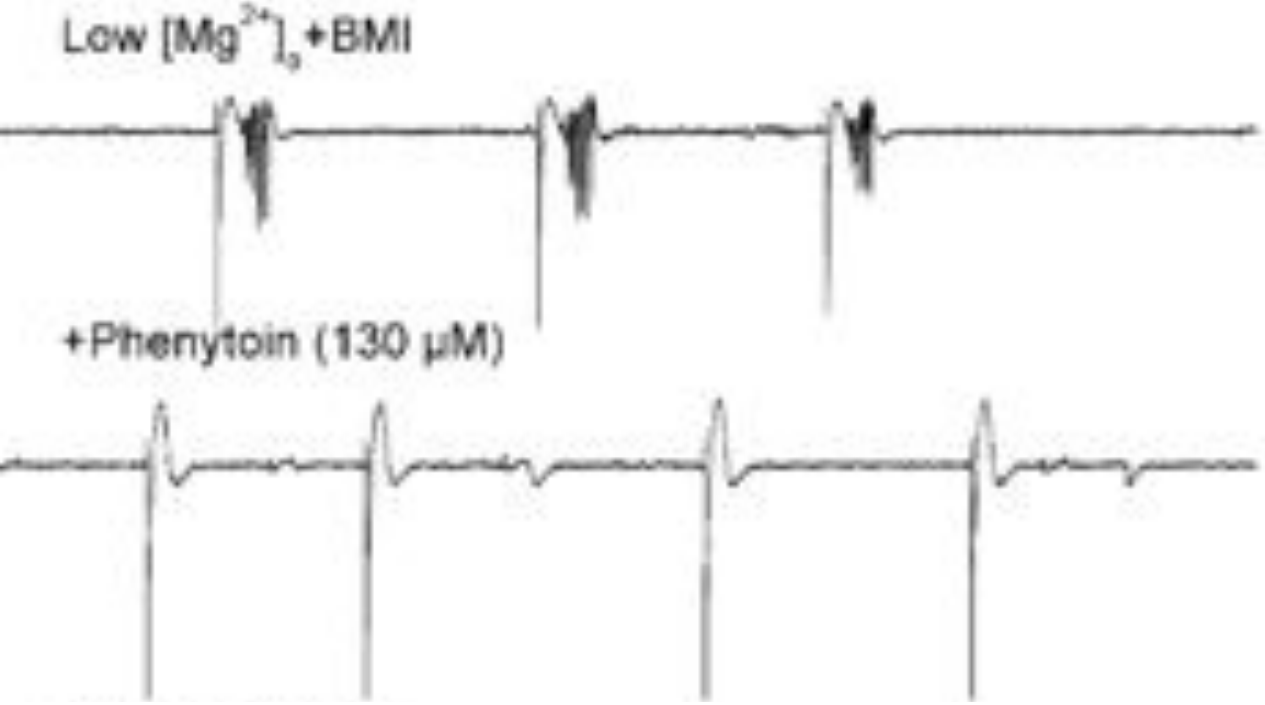
Local Field Potential



Multi Unit Activity

Neuron

Pharmacology of interictal- and ictal-like epileptiform activity in vitro.

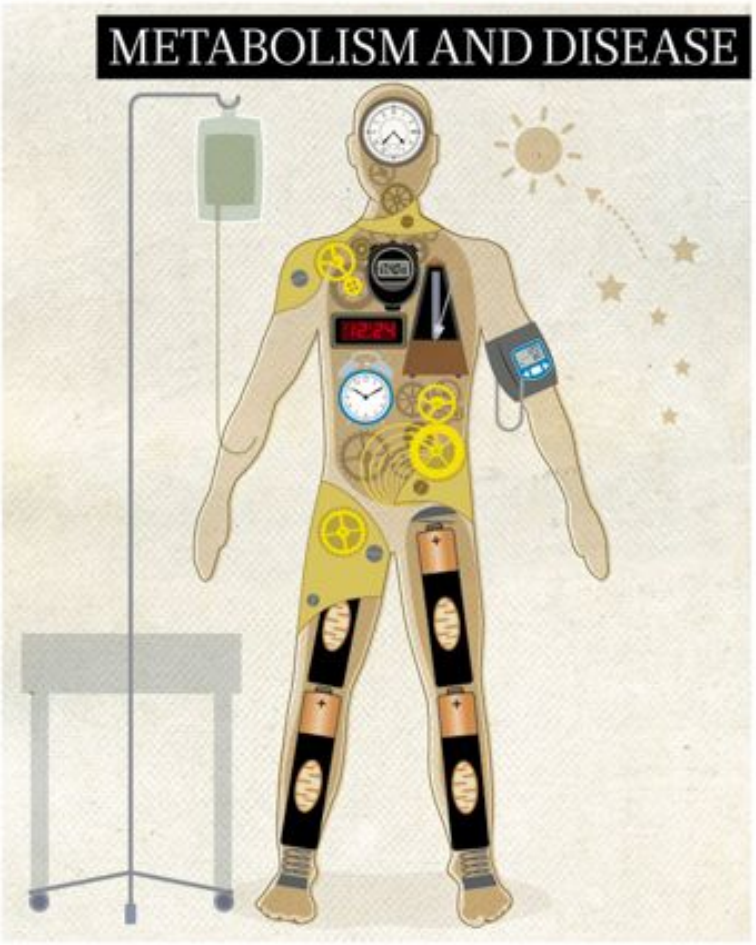


Mouse metabolism facility: physiology and pathophysiology

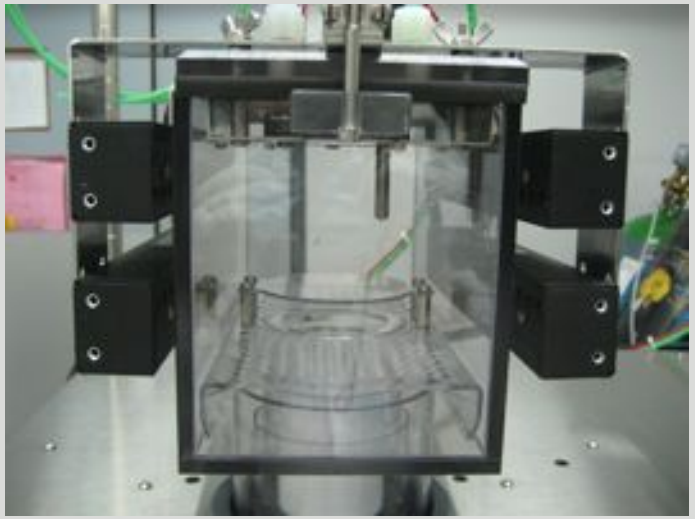
WHY ?

Altered metabolism is at the heart of serious health problems such as

- **obesity**
- **diabetes**
- **CVDs**
- **cancer**
- **hypertension**
- **endothelial dysfunction**
- **oxidative stress**
- **inflammation**
- **fatty liver disease (NASH)**



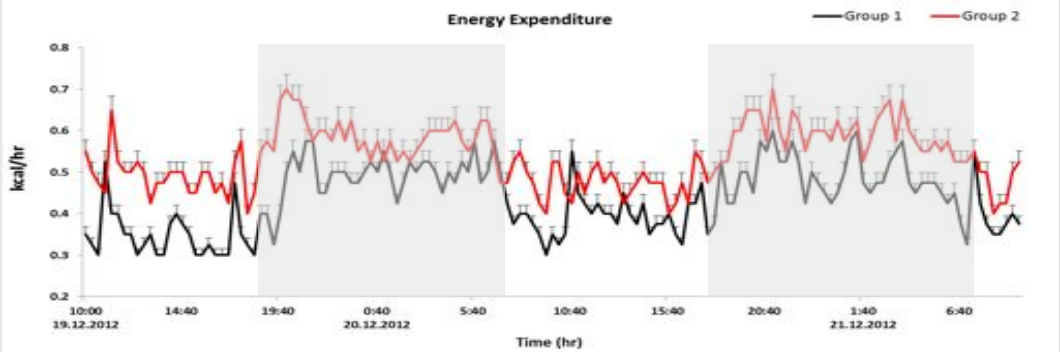
Comprehensive Lab Animal Monitoring System (CLAMS)

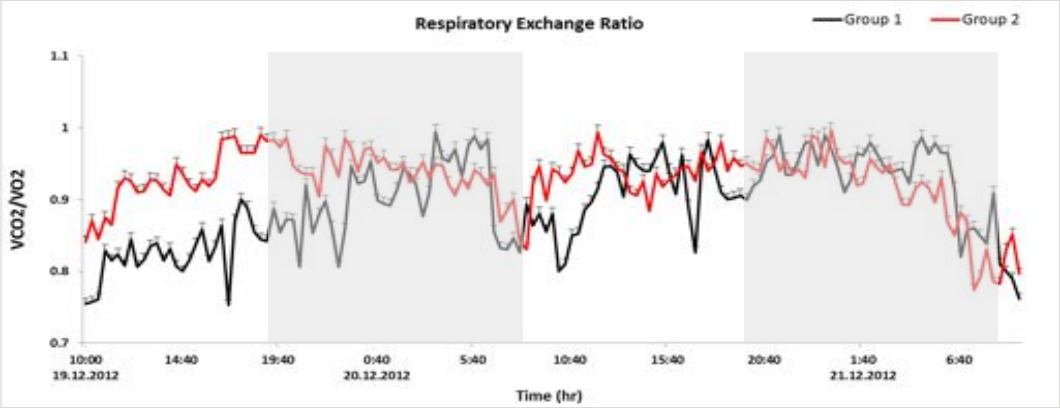


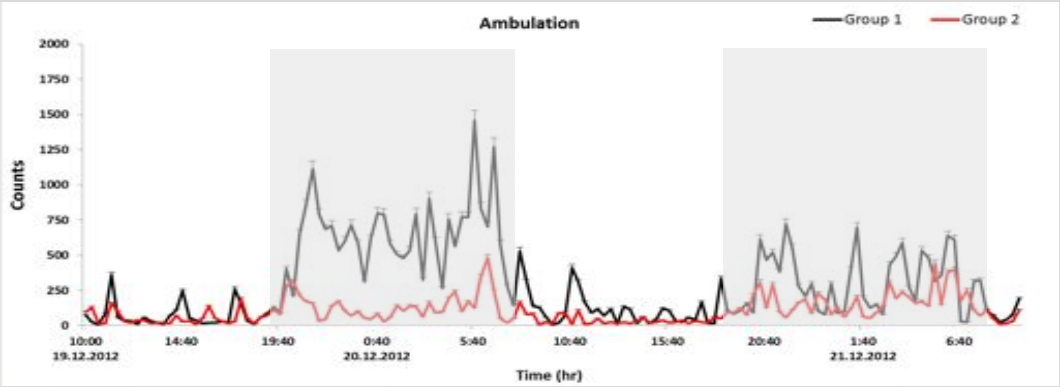
- Open circuit indirect calorimetry system that provides high quality metabolic phenotyping analysis

- Metabolic Measurements:
  - Energy expenditure
  - Respiratory Exchange Ratio (RER)
  - Feeding measurements
- Activity measurements:
  - Rearing axis **(ZTOT)**
  - Ambulatory **(AMB)**

CLAMS Analysis





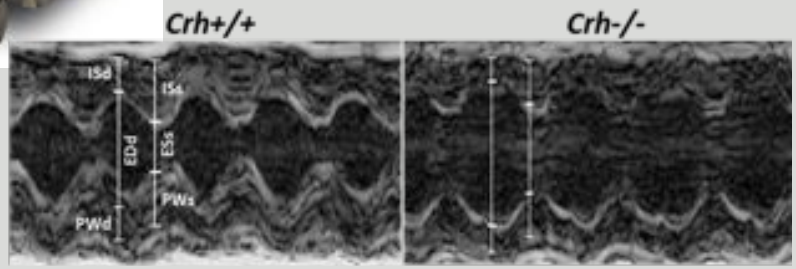


Cardiovascular function

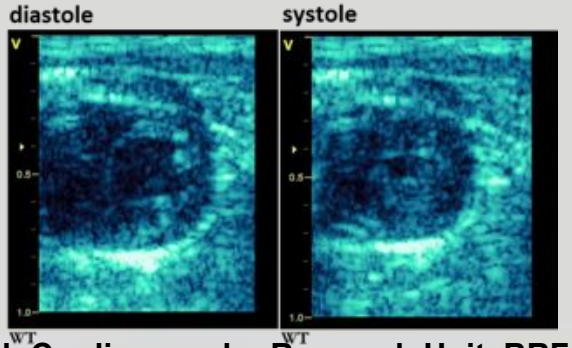
Functional assessment

Two-D M-mode echocardiography

Vivid 7, GE Healthcare system

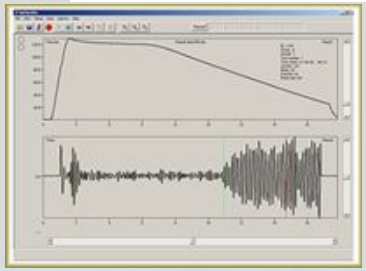


Echopac PC SW 3.1.3 software (GE Healthcare)



(In collaboration with Cardiovascular Research Unit, BRFAA)

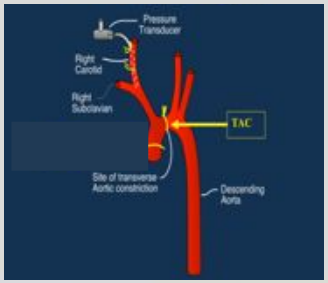
Blood pressure measurement system



Applications of Metabolic phenotyping from our studies so far….

Mouse Models of Human Diseases

- *Obesity, insulin resistance*
- *Diabetes*
- *Degenerative CNS disorders*
- *Infection, systemic inflammation*
- *Cardiovascular dysfunction*
- *Induced ischemia (TAC)*



- *Drug effects (IL15, aDllr4, Isoproterenol, metformin, rapamycin, insulin, PPAR ligands)*

and side effects…

In-Silico modeling studies of *in vitro* and *in vivo experiments*

General Concepts and Objectives:

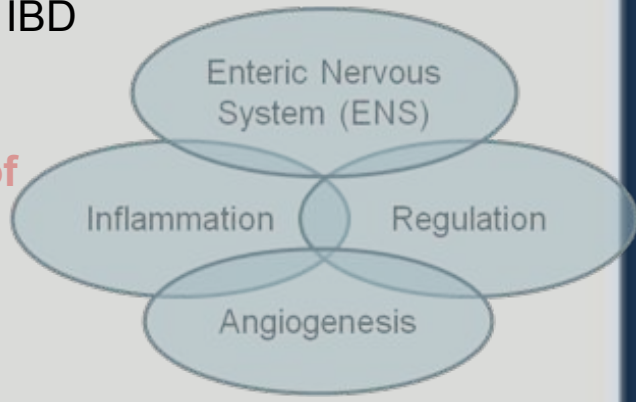
- Develop basic model in order to capture the biological knowledge from biological studies.
- Suggest new experiments and feed the results back to the model to improve it.
- Gradually introduce and integrate more components into the model
- Build a computational platform where new hypotheses could be tested *in silico*

In-Silico modeling studies of the immune modulatory mechanisms in Inflammatory Bowel Disease

Concept:

Inflammatory Bowel Disease results from mis-communication between gut commensal flora and the immune system of the host

The **ENS** is increasingly recognized as a critical component in IBD



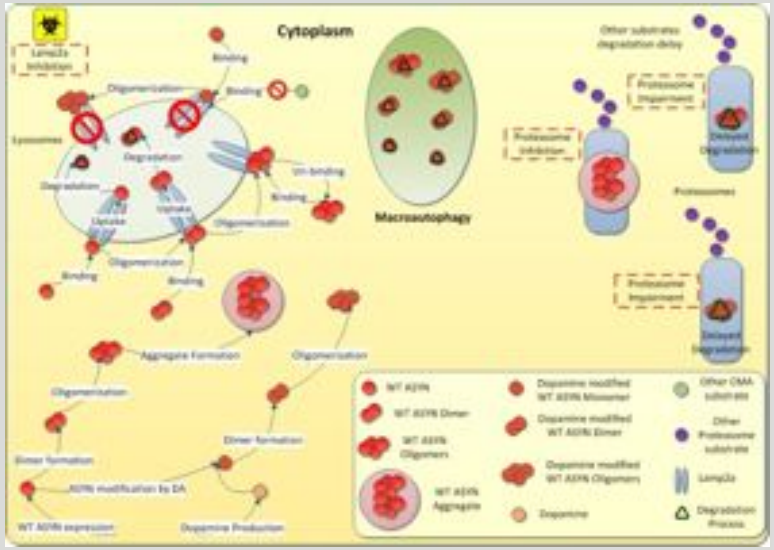
Objectives:

- Create the **first multi-component computational model of IBD** from mouse and human studies.
- Elucidate the contribution of cell subsystems in the restitution of the epithelial barrier.
- Observe cell behaviors in-silico and generate new testable hypotheses.
- Identify specific parts that could likely be manipulated and suggest new therapeutic approaches for human disease.

In silico modeling of Alpha-synuclein oligomerization effects on neuronal homeostasis

Concept:

•Duplications and/or mutations of the ASYN gene are linked with familial PD, suggesting that ASYN levels are crucial for neuronal homeostasis.
Modification of ASYN by Dopamine is crucial for homeostatic dysregulations of neurons



Objectives:

Create a mathematical model aiming to:

•Simulate the dynamics of ASYN (over)expression oligomerization and the basic biological pathways involved in the aberrant function of intracellular ASYN.

•Unravel the unknown critical balance in the levels of intracellular ASYN necessary to cause such homeostatic dys-regulations

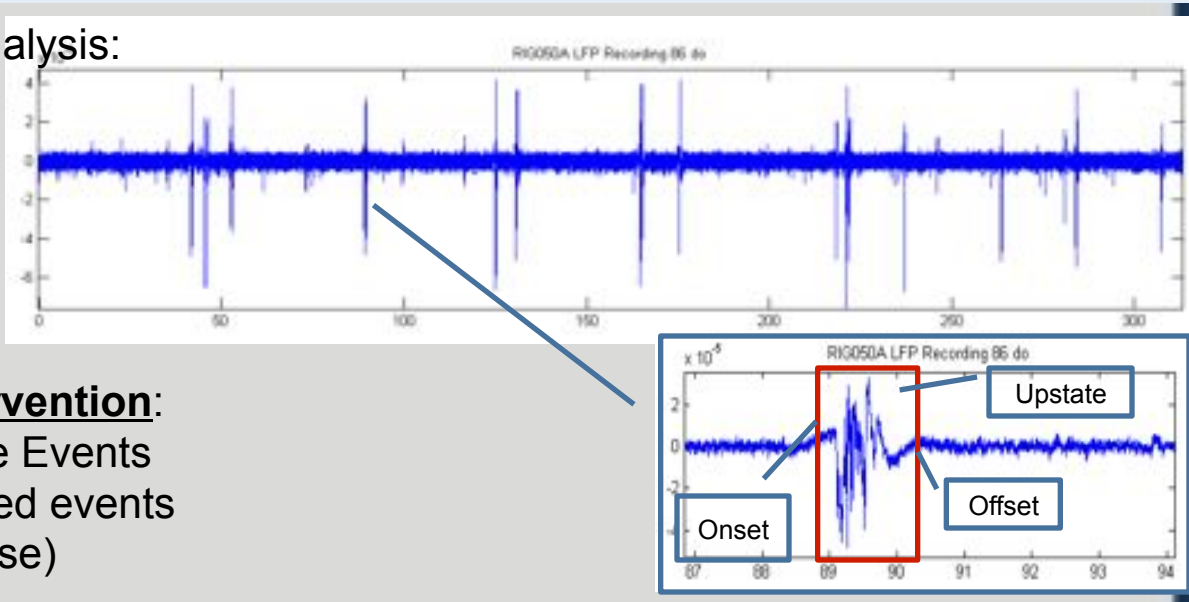
•Combine and compare biological knowledge from different biological labs

Electrophysiology - Time Series Signal Analysis

➢**Automated** Event Detection and Analysis:

Release user **AND** data from making biased estimations!!!

The software adapts to the dynamic change of the recording during the recording…



➢ **Additional Properties - User Intervention**:

✓ allow user to modify the limits of the Events

✓On-line Quality control of the detected events (discard any mechanical/electrical noise)

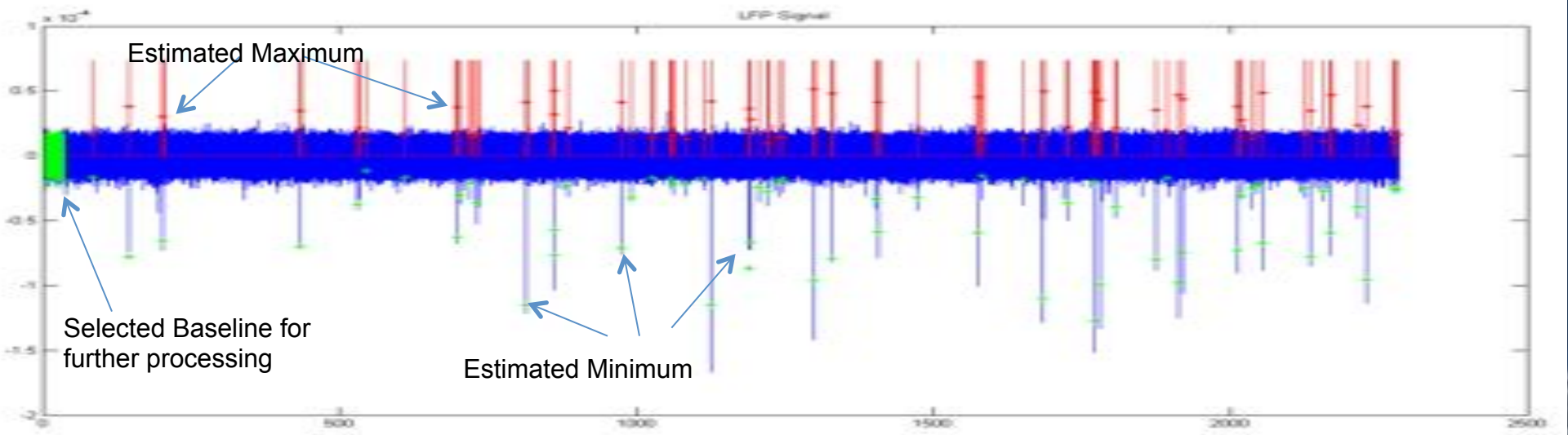


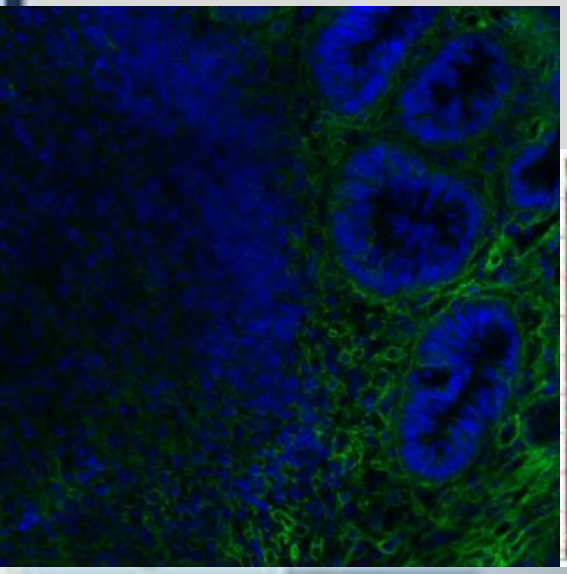
Image Analysis & Quantification…

Aim:
Development of specialized software for Image Analysis and Quantification

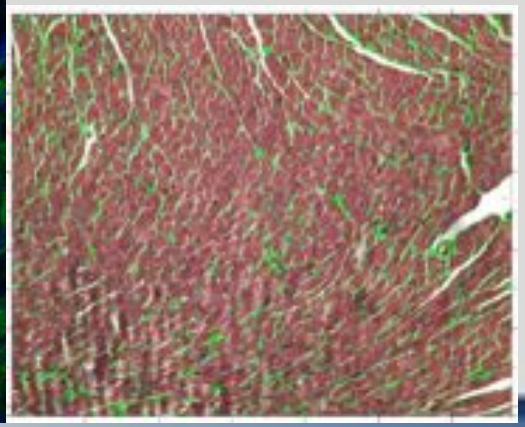
Developed Software Properties:

1. Fully Automated.
2. High Throughtput Analysis, in order to release users and data from biases.
3. Export the Quantification results in Excel files for further analysis.
4. Applications on Confocal Images, Histological Images, cell cultures and/or videos.

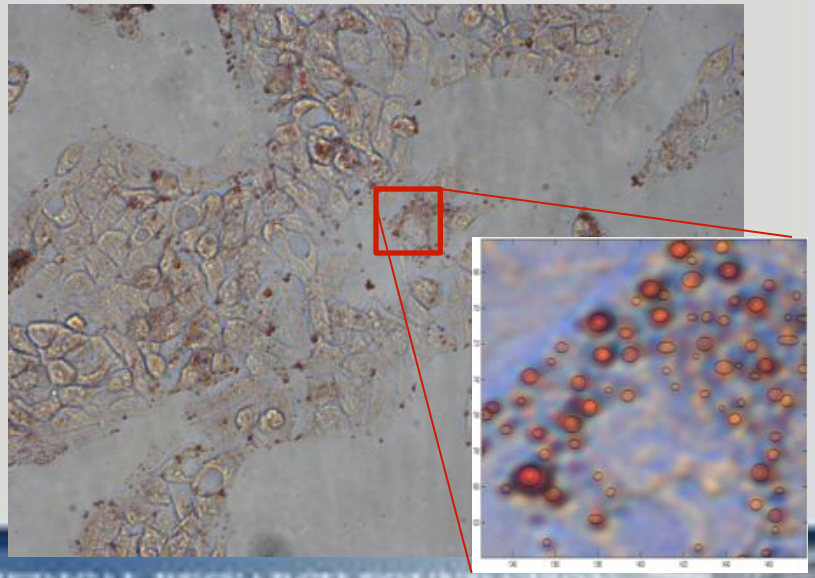
Confocal Images



Histological Images
Masson's staining



Cell Culture – Oil Red O Staining

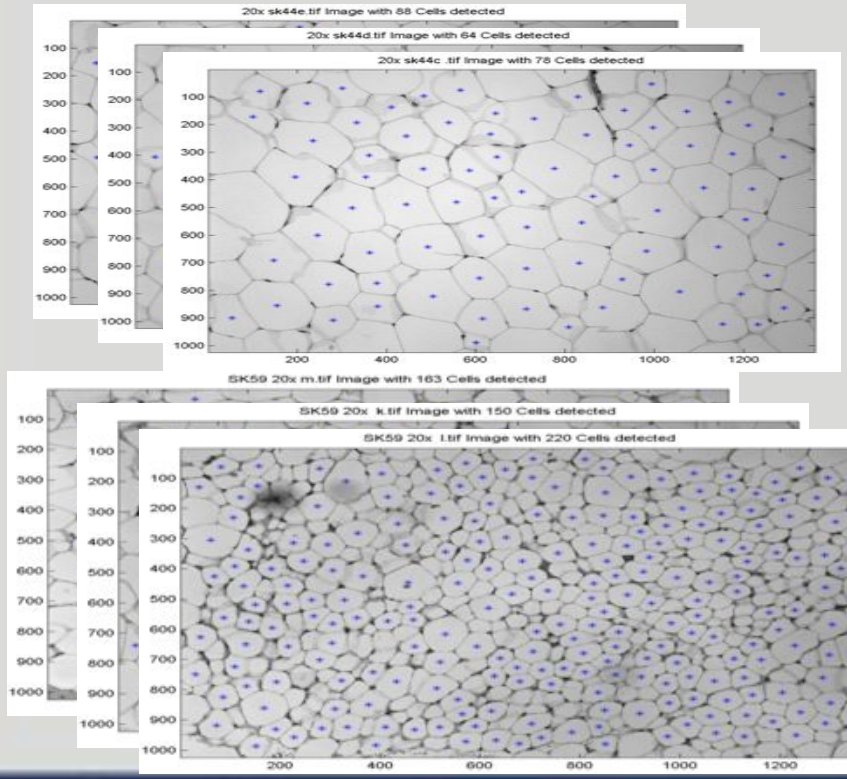


Adipocyte Quantification and Analysis…

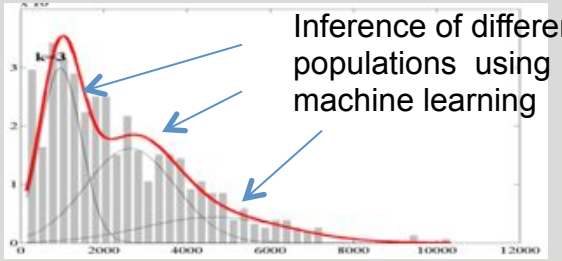
Aim: Enumerate and quantify the area of individual adipocytes. Characterize the distributions of cells area in different biological groups.

Developed Software:

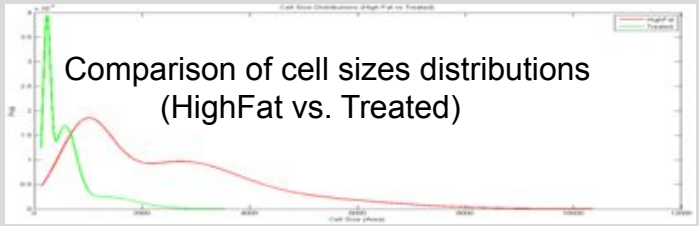
Fully automated, results exported in Excel files, user specific distributions.



Fitting of Gaussian Mixture on the Cell Size Distribution

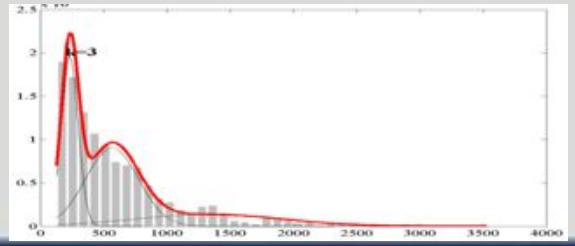


Inference of different cell populations using machine learning



Comparison of cell sizes distributions (HighFat vs. Treated)

Fitting of Gaussian Mixture on the Cell Size Distribution

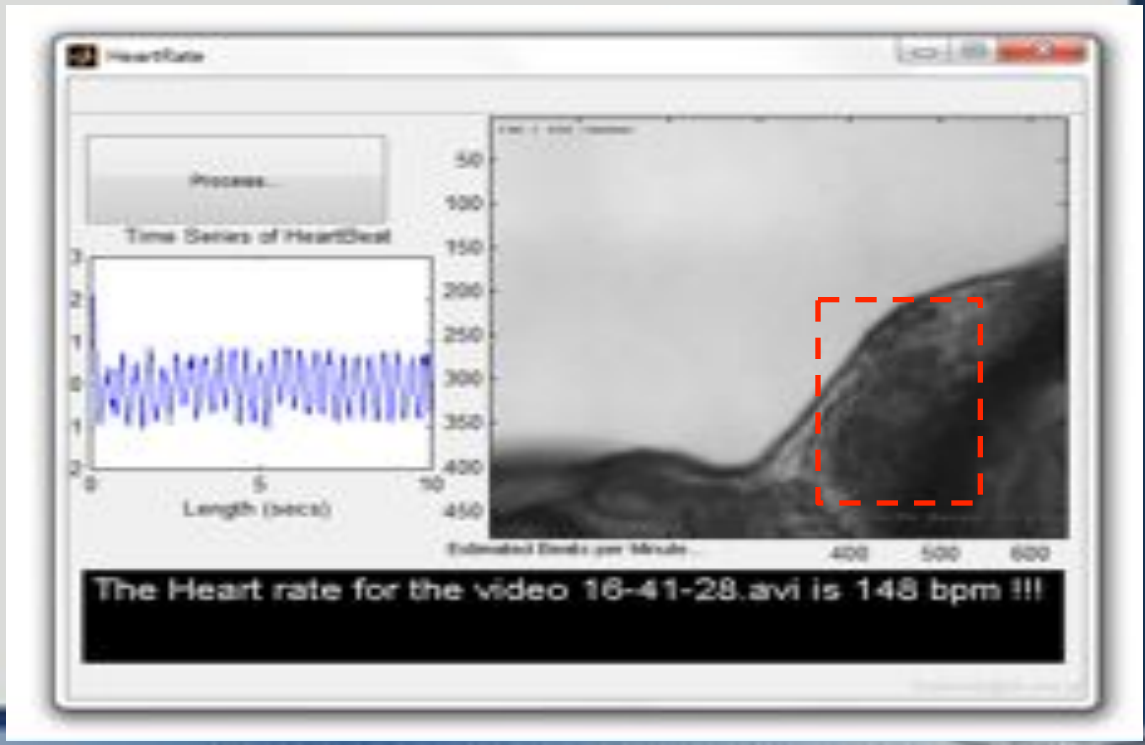


ZebraFish Heart Rate estimation from videos…

- Input the video (user)
- Delineate the heart area (approximately) (user)
- The corresponding Heart Rate is displayed along with the estimated signal of the heart-beat (automated)
  - ✓Find the fluctuations of the intensity in the area of interest (time-series).
  - ✓Fourier analysis in order to find the dominant frequency (Heart Rate).

Robust to:

1. small animal movements
2. small light variations during video acquisition
3. limited (in terms of time) accidental movement by the user

Stand-alone application (2 versions):

1. One video at a time analysis.
2. A series of video exporting results in Excel
   The heart must be approximately at the same site for all videos.